Oxidation-responsive Magnetic Nanostructure-loaded Bicontinuous Nanospheres for Drug Delivery

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Statement of Purpose: Magnetic nanostructures (MNS) have a multitude of applications due to their superparamagnetic properties[1]. To maximize these biologic applications, challenges like stability, inability to encapsulate therapeutics, and variable clearance rates *in vivo* must be overcome. Bicontinuous nanospheres (BCNs) of poly(ethylene glycol)-*b*-poly(propylene sulfide) (PEG-*b*-PPS) copolymer allow effective encapsulation and controlled delivery of both hydrophilic and hydrophobic cargo due to their cubic architecture[2]. To enhance the utility of MNS for biologic applications, we report the development and characterization of oxidation-responsive, MNS-embedded BCNs (MBCNs) co-loaded with diverse molecular payloads for sustained drug delivery.

Methods: Flash nanoprecipitation (FNP) was used to form MBCNs via loading 4 nm metal ferrite MNS into PEG_{17}-*b*-PPS_{75} BCN polymer. Dynamic light scattering (DLS) and cryoTEM were used to study MBCN size and structure. MNS and small molecule loading was measured by inductively-coupled plasma mass spectrometry (ICP-MS) and UV-VIS. ICP-MS was used to study MBCN organ biodistribution 4 h, 24 h, and 7 d post IV administration in Balb/CJ mice. Confocal microscopy was used to study *in vitro* uptake. Oxidation responsiveness was studied using TEM following MBCN incubation at 5 M H_2O_2 for 4 h.

Results: Hydrophobic 4 nm MNS were stably loaded into PEG-*b*-PPS BCNs to form MBCN via FNP (Fig 1a). MBCN retained the same size distribution and cubic architecture of blank BCN[2] (Fig 1b). MBCN simultaneously encapsulated hydrophilic and hydrophobic cargo, as well as model drugs (Fig 1c), with concurrent high MNS encapsulation efficiency. As further proof of MNS encapsulation, MBCNs demonstrated higher r_2 relaxation rates compared with free MNS (Fig 1d), likely due to MNS clustering[1] within MBCNs and demonstrating MRI contrast potential. Intracellular delivery of multiple payloads via MBCNs was demonstrated using DiD and FITC-BSA as model drugs, with both payloads co-localizing with lysosomes in MCF7 cells (Fig 1e). Like other PEG-*b*-PPS nanocarriers[3,4], MBCNs were found to be oxidation-responsive, undergoing a transformation to MNS-loaded micellar structures in oxidative conditions (Fig 1f). To the best of our knowledge, this is the first report of nanoscale payload transfer from one nanocarrier morphology to another, and the first demonstration of *in situ* MNS-loaded micelle generation.

Conclusions: MBCNs were formed via encapsulation of MNS and diverse molecular payloads within PEG-*b*-PPS BCN polymer. MBCNs were able to deliver these diverse payloads intracellularly and demonstrated enhanced r_2 relaxivity. Finally, MBCNs displayed a unique oxidation-responsive morphological transition to MNS-loaded micelles, making them an intriguing platform for future sustained delivery applications.

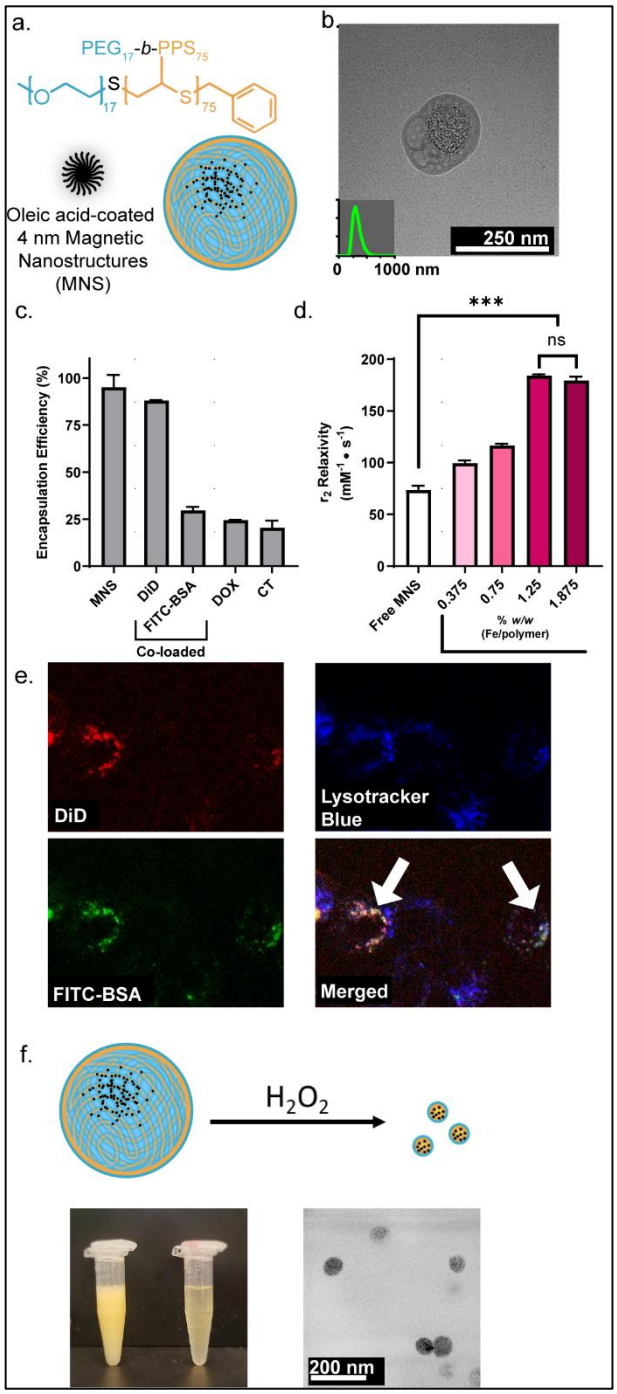

Figure 1. MNS-loaded BCN[5] a) schematic, b) physicochemical characterization, c) payload encapsulation efficiency, d) relaxivity rate analysis, e) *in vitro* payload delivery, and f) oxidative transition schematic, photographs, and TEM analysis.

References: 1. Nandwana V. ACS Appl Mater Interfaces. 2016; 8:6953-6961. **2.** Bobbala S. Nanoscale. 2018; 10:5078-5088. **3.** Karabin N. Nature Comm. 2018; 9:624. **4.** Bobbala S. Nanoscale. 2020; 5332-5340. **5.** Modak M. ACS Appl Mater Interfaces. 2020.